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Microtiter Determination of Measles Hemagglutination Inhibition Antibody with Filter Papers

JAMES H. NAKANO,[1]* DONNA L. MILLER,[1] STANLEY O. FOSTER,[2] AND EDWARD W. BRINK[3]

Division of Viral Diseases, Center for Infectious Diseases,[1] International Immunization, International Health Program Office,[2] and Immunization Division, Center for Prevention Services,[3] Centers for Disease Control, Atlanta, Georgia 30333

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Epidemiological studies of measles and measles immunization frequently require determination of measles antibody status. In developing countries, where venipuncture is frequently unacceptable and where refrigerated storage of serum specimens is often unavailable, microtiter techniques not requiring refrigeration are required. We developed a filter paper technique that measures measles hemagglutination inhibition antibody and meets these criteria. Comparison of separately collected venous blood and peripheral blood collected on filter paper demonstrated 97% agreement in terms of presence or absence of antibody. In 30 of 32 measles specimens, 94% of titers were the same or varied by less than 2 twofold dilutions.

Measles is a major cause of childhood mortality worldwide. In Africa, where the disease is frequently severe, 13 million cases and 650,000 deaths are estimated to occur annually. Deaths are usually the result of complications: pneumonia, diarrhea, undernutrition, or some combination of the three.

Measles vaccine was first used in Africa in 1962 (13). Mass campaigns in the 1960s had spectacular results in stopping ongoing measles transmission (4). Problems in strategy, vaccine storage, and vaccine delivery have, however, limited the ability to maintain measles control. In 1974, a study by McBean et al. (12) showed that only 40% of susceptible persons receiving measles vaccine are effectively immunized. Improvement in vaccine quality, vaccine storage, supervision, and training has increased the effectiveness of vaccine delivery (14).

To document this progress and to better identify populations at risk and the effectiveness of measles vaccination, investigators need effective serological survey techniques. Because most studies are carried out in the field where refrigeration is not available and where venipuncture is not well accepted, a microtiter technique not requiring refrigeration is needed.

Early reports (1, 2, 3, 7, 8) of methods for collecting blood by filter paper used filter paper disks adapted for antibiotic sensitivity tests. The disk was held by forceps to a fingertip puncture and saturated with whole blood. Each blood-saturated disk was stored in a separate container to keep its identity. Difficulties in obtaining a standard quantity of blood on each disk and in drying without bacterial contamination limited the usefulness of this technique in field situations. To overcome these difficulties, we adapted a filter paper technique originally developed for malaria by Matthews et al. (10, 11) for measles hemagglutination inhibition (HI). HI antibody was determined by the methods of Hierholzer and Suggs (5, 6).

MATERIALS AND METHODS

Filter paper. Filter paper ROPACO 1023.038 was purchased from the Rochester Paper Co. (Rochester, Mich.). A sheet (20 by 20 inch [ca. 51 by 51 cm]) was cut into rectangles (1 by 3 inch [ca. 2.5 by 7.6 cm]), and each rectangle was imprinted with two circles, each 12 mm in diameter. The unmarked area of the filter paper rectangle (see Fig. 1, filter paper sample) was used to write identifying information such as name, date of collection, and study group or geographical location.

Collection of blood on filter paper. A sample of blood was collected from a finger or heel puncture by placing the underside of each of the imprinted circles on the bleeding site. The blood soaked through the paper until each circle was completely filled. The filter papers were placed inside slide collection boxes and dried at ambient temperature. Drying reduced microbiological contamination. When the blood spots were dried, the rectangles were bundled with a rubber band and stored in plastic bags at ambient temperature, or at 4 to 8°C or −10°C if cooling equipment was available, until shipped. In a high-humidity environment, silica gel was added in the storage bag to promote adequate drying, which was important to discourage the growth of contaminating organisms on specimens stored at ambient temperatures. The specimens were shipped with wet ice to the Centers for Disease Control, Atlanta, Ga. Transportation usually took 2 to 4 days. The specimens received were stored at −20°C until the blood was extracted from the filter papers and assayed for HI antibody.

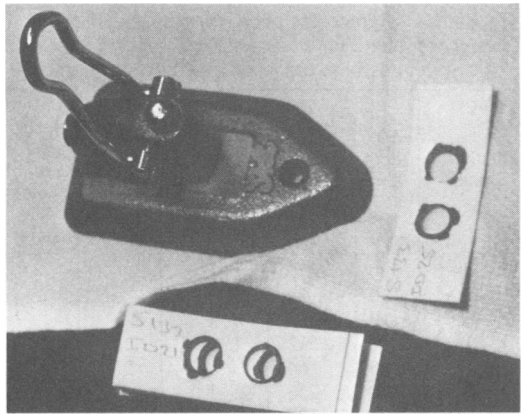

FIG. 1. Paper punch which cuts 10-mm-diameter disks.

Punching filter paper for test samples. The frozen filter paper with the absorbed specimens was warmed, and the two blood-soaked circles on the rectangle were punched out by a paper punch (Fig. 1) made by Wilson Jones Co. (number 118-4 [stock number 59]; Chicago, Ill.) with a punch diameter of 10 mm or mole size 13/32. The punched-out disks were examined, and only those that were completely covered with blood were used.

Extraction of blood from disks. The two blood-soaked disks punched out from each filter paper rectangle (one rectangle for each patient) were placed in a well of a previously labeled disposable plastic tray (Linbro Dispo-Trays, model 96U-CS, containing 96 wells; Linbro Div., Flow Laboratories, Inc., Hamden, Conn.) (Fig. 2). To each well containing two disks, we added 0.5 ml of phosphate-buffered saline (PBS), pH 7.2. The disks were soaked in the PBS for 30 min at room temperature. During this period, the disks were agitated by a broken applicator stick, using a fresh stick for each well. The fluid from the two disks in each well were expressed by a long-nosed, 6.5-inch (ca. 16.5-cm), smooth-jawed, duckbilled plier into their respective well by squeezing the disks between the jaws and at the same time touching the side of the well with the jaws so that the fluid could flow from the jaws into the well (Fig. 2). Each well contained a dark-brown eluate which was composed of the eluted immunoglobulin as well as degraded hemoglobin.

Calculation of approximate dilution. (i) Empirical method. Mathews (10) compared indirect hemagglutination assay results of blood collected by filter paper and those of serum of the same person's blood collected by venous puncture. He compared the titers of many paired specimens and concluded that an extract from one filter paper disk eluted with 0.4 ml of PBS constituted a dilution of approximately 1/16 of that contained in undiluted serum from the venous blood. On the basis of this, we calculated that the extract from one filter paper disk soaked in 0.5 ml (instead of 0.4 ml) of PBS was diluted approximately 1/20. However, since this dilution was judged to be too high as a starting dilution to detect measles HI antibody titers

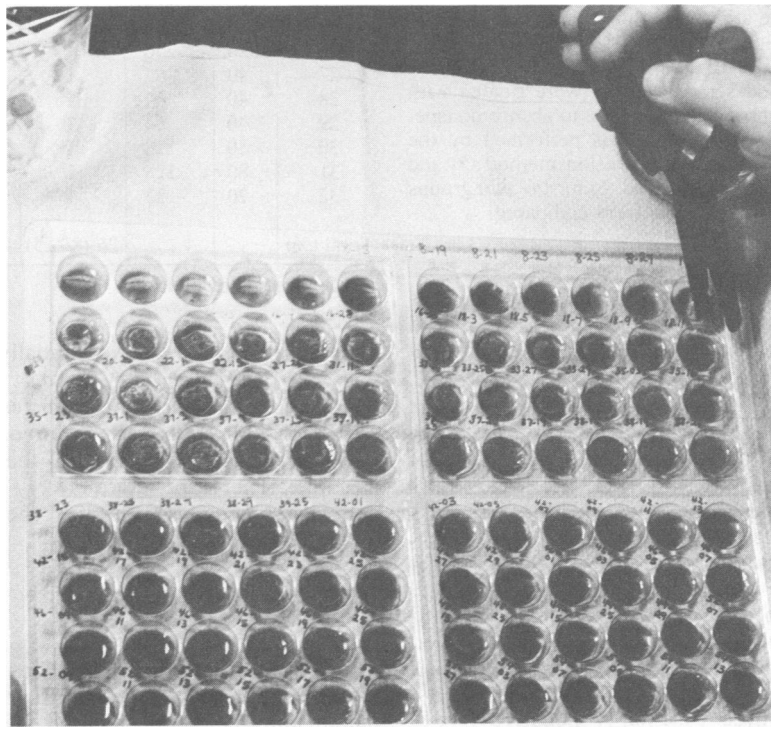

FIG. 2. Plastic 96-well tray with blood-soaked filter paper disks in PBS. Note the duckbilled, smooth-jawed plier used to extract the blood.

between 1/10 and 1/20, we used two filter paper disks soaked in 0.5 ml of PBS (pH 7.2). This increased the starting dilution to 1/10.

(ii) Globulin quantitation method. Venous blood collected with an anticoagulant was loaded onto the paired circles on each of 10 filter paper rectangles. When the blood spots were thoroughly dried, two 10-mm disks of blood spots were punched out of each rectangle. Each pair of disks (a total of 10 pairs) was soaked in 0.5 ml of PBS (pH 7.2), and the 10 eluates were pooled. A sample from this pool and a sample of the uncoagulated whole blood were treated with ammonium sulfate (final concentration, 35%) to precipitate the globulin content of each sample. The precipitated globulins from the eluate and from the whole blood were dissolved, and the proteins were assayed by the Lowry method (9). The protein content in the eluate globulin was found to be 6.95 mg/ml, and in the whole blood, 65.2 mg/ml. The ratio of the globulin protein content of the eluate to that of the whole blood was 1/10.95. Thus, 1/10.95 of the globulin content in the whole blood was found in the eluates, or the eluate is a 1/10.95 dilution of the whole blood.

(iii) Volumetric method. The volume of whole blood required to load one 10-mm punched-out filter paper disk under simulated field conditions was 0.05 ml. Two disks, each containing 0.05 ml of dried whole blood soaked in 0.5 ml of PBS, gave an eluate with a whole-blood dilution of 0.1 to 0.5 or 1/5. If the serum portion of the whole blood was assumed to be about 60%, however, the dilution of the serum would be 0.06 to 0.5 or 1/8.3.

In summary, the antibody dilution estimated by the three methods ranged from 1/8.3 to 1/10.95. From these results, we arbitrarily chose 1/10 as an approximate starting dilution.

Assay of measles HI antibody. The eluted samples and venous blood serum samples were treated with 50% vervet erythrocyte suspension to absorb nonspecific agglutinin. The HI test was performed by the standardized erythrocyte suspension method (5) and by the standardized HI method (6) for the two groups of samples, and their results were compared.

RESULTS

The measles HI results for eluates and venous blood sera of 32 African children are compared in Table 1. The starting dilution of the eluate was 1/10 and of the venous blood sera was 1/8.

Twenty-six (81.25%) sets of specimens (filter paper blood and serum) had titers differing by less than 1 twofold dilution (less than one well of the microtiter plate); four (12.5%) had titers differing by less than 2 twofold dilutions (less than two wells); and two (6.25%) had titers differing by greater than 2 twofold dilutions. Because the test is primarily used for serological surveillance to determine the presence of antibody at a 1/10 dilution, the agreement between the results obtained by testing filter paper blood and venous blood is 97% (31 of 32).

There are three essentially negative (<1/8) results for the serum testing (individuals number 3, 10, and 21), and the corresponding results for the filter paper blood are essentially negative for two (<1/10), whereas the third is 1/10 (number 21) (Table 1). Thus, for serum testing, 3 of 32 (9.4%) of the results were negative, and for filter paper blood, 2 of 32 (6.3%) of the results were negative. Therefore, there is very little concern for false-positive results for the filter paper blood test.

TABLE 1. Agreement between measles HI titers of peripheral blood collected on filter paper and serum of venous blood from individuals in Africa

Individual	HI titers (filter paper)	HI titers (serum)[a]	Specimens less than ±1 twofold dilutions	Specimens less than ±2 twofold dilutions	Specimens greater than ±2 twofold dilutions
1	320	256	1		
2	40	32	1		
3	<10	<8	1		
4	160	≥512		1	
5	80	64	1		
6	320	256	1		
7	320	128		1	
8	80	32		1	
9	80	64	1		
10	<10	<8	1		
11	40	64	1		
12	320	≥512	1		
13	80	16			1
14	160	64		1	
15	160	128	1		
16	20	16	1		
17	40	32	1		
18	640	≥512	1		
19	640	64			1
20	20	16	1		
21	10	<8	1		
22	20	32	1		
23	80	64	1		
24	160	128	1		
25	160	128	1		
26	320	256	1		
27	40	64	1		
28	40	64	1		
29	40	64	1		
30	80	128	1		
31	80	128	1		
32	20	32	1		
Total (%)			26 (81.25)	4 (12.5)	2 (6.25)

[a] Reciprocal of dilution.

DISCUSSION

Several viral antibody assays, including measles HI, described in the previous reports used filter paper blood collection. However, the filter paper blood collection method in these reports used individual disks to absorb the peripheral blood. This meant that each disk was manipulat-

ed by forceps to absorb the blood and that the blood-soaked disk for each individual had to be placed in a separate container to keep its identity. In a separate container, drying the blood-soaked disks to reduce microbial growth was also a problem unless the blood-soaked disks were always frozen or treated with an antimetabolite. However, the blood collected by our method can be identified by names or numbers written in the unmarked area of the rectangular filter paper, laid out to dry, and then bundled and stored in a plastic bag containing a dessicating agent and stored at ambient temperatures or in a refrigerator or a freezer until shipped.

Since Mathews (10) reported sufficient agreement between indirect hemagglutination assay titers of filter paper blood and venous sera, we can also conclude that the two groups of specimens that we tested for measles HI titers agreed sufficiently for the screening of positive measles antibody. We consider that this method of blood collection is dependable for measles HI surveillance in Africa and that it can be substituted for the more cumbersome and often impossible method of venous blood collection from children in the field studies in many developing countries.

Recently, this method has been recommended in the United States to facilitate measles surveillance during the period of measles elimination.

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